This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2016.066a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Agrican357virus*, including five (5) new species in the family *Myoviridae*.
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☒

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List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	ICTV Bacterial and Archaeal Viruses Subcommittee

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV: July 2016
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2016.066aB***	(assigned by ICTV officers)

To create 5 new species within:

Genus:	***Agrican357virus* (new)**
Subfamily:	
Family:	***Myoviridae***
Order:	***Caudovirales***

Fill in all that apply.
- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Erwinia virus RAY	Erwinia phage vB_EamM_RAY	KU886224
Erwinia virus Simmy50	Erwinia phage vB_EamM_Simmy50	KU886223
Erwinia virus Deimos	Erwinia phage vB_EamM_Deimos-Minion	KU886225
Erwinia virus SpecialG	Erwinia phage vB_EamM_Special G	KU886222
Erwinia virus Ea35-70	Erwinia phage Ea35-70	KF806589

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
    - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
    - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2016.066bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Myoviridae***
Order:	***Caudovirales***

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2016.066cB***	(assigned by ICTV officers)

To name the new genus: *Agrican357virus*

Assigning the type species and other species to a new genus

Code	***2016.066dB***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Erwinia virus Ea35-70

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

5

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

Erwinia phage Ea35-70 was isolated from soil samples collected under an infected pear tree in southern Ontario, Canada in 2013, while the remainder of the phages were isolated more recently in Utah (USA). In all cases *Erwinia amylovora* strains were used as the host [5, 6]. As pointed out by Yagubi et al. [5] phage Ea35-70 is peripherally related to the *Phikzvirus* genus.

BLASTN, CoreGenes (Table 1, Fig. 2) [3,4], phylogenetic analyses (Fig. 3) [1], and progressiveMAUVE alignments (Fig. 4) [2] all indicate that the proposed genus, *Agrican357virus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 272.0 kb in length (49.9 mol% G+C), and encode 320 proteins and 0-1 tRNAs.

Origin of the new genus name:

The name is derived from Agriculture and Agri-Food Canada (Vineland) where the first phage of this type was isolated and sequenced. Since all of their viruses are named "Ea…", we had the potential, as with "Phi" of a number of genera beginning with "Ea."

Reasons to justify the choice of type species:

The first sequenced member of this genus.

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008; 36(Web Server issue):W465-9.
2. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147.
3. Agren J et al. (2012) Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One.;7(6):e39107
4. Turner D, Reynolds D, Seto D, Mahadevan P. CoreGenes3.5: a webserver for the determination of core genes from sets of viral and small bacterial genomes. BMC Res Notes. 2013;6:140.
5. Yagubi AI, Castle AJ, Kropinski AM, Banks TW, Svircev AM. Complete Genome Sequence of *Erwinia amylovora* Bacteriophage vB_EamM_Ea35-70. Genome Announc. 2014;2(4). pii: e00413-14.
6. Gill JJ, Svircev AM, Smith R, Castle AJ. 2003. Bacteriophages of *Erwinia amylovora*. Appl. Environ. Microbiol. 69:2133–2138.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Fig. 1. Electron micrograph of negatively stained Erwinia phage SpecialG (provided by Dr. Julianne H. Grose, College of Biological Sciences, Brigham Young University, Provo, Utah, USA)

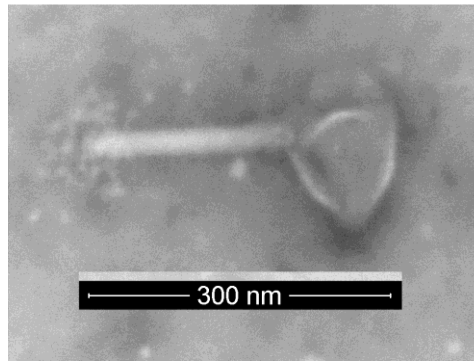

Fig. 2. BLASTN analysis of all these viruses using Gegenees [3] with "sensitive" settings of fragmenting algorithm - size: 200 bp, shift 100 bp. The results were exported to Excel and the heatmap is colored according to percentage identity (>70% green, >80% yellow, >95% red). Strains belonging to the same proposed species are boxed in black.

PHAGE	Deimos-Minion	SpecialG	Simmy50	RAY	Ea35-70
Deimos-Minion	100.0	90.2	90.4	90.7	90.2
SpecialG	90.3	100.0	92.6	92.7	89.7
Simmy50	91.1	93.3	100.0	94.3	90.6
RAY	91.5	93.3	94.3	100.0	91.3
Ea35-70	91.0	90.5	90.5	91.3	100.0

Table 1. Properties of phages belonging to the genus *Agrican357virus*.

Erwinia phage	RefSeq No.	GenBank No.	Genome length (kb)	GC%	Protein	tRNA	% DNA Sequence Identity*	% Protein Sequence Identity**
Ea35-70	NC_023557	KF806589	271.08	49.9	318	1	100.0	100.0
RAY		KU886224	271.18	49.9	317	1	91.3	91.1
Simmy50		KU886223	271.09	49.8	319	1	90.6	97.8
SpecialG		KU886222	273.24	49.8	323	0	89.7	98.4
Deimos		KU886225	273.50	49.9	325	0	90.2	98.4

* determined using Gegenees BLASTN: ** determined using CoreGenes3.5

Fig. 3. Phylogenetic analysis of (A) major capsid proteins and (B) large subunit terminase proteins of a variety of Erwinia and related phages constructed using "one click" at phylogeny.fr [1]. "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is already set up to run and connect programs recognized for their accuracy and speed (MUSCLE for multiple alignment and PhyML for phylogeny) to reconstruct a robust phylogenetic tree from a set of sequences." It also includes the use of Gblocks to eliminate poorly aligned positions and divergent regions. "The usual bootstrapping procedure is replaced by a new confidence index that is much faster to compute.

See: Anisimova M., Gascuel O. Approximate likelihood ratio test for branches: A fast, accurate and powerful alternative (Syst Biol. 2006;55(4):539-52.) for details." The TerL from Gaia contained a intein which was removed before the phylogenetic tree was constructed. Agrican357viruses are boxed in red.

A. Major capsid proteins

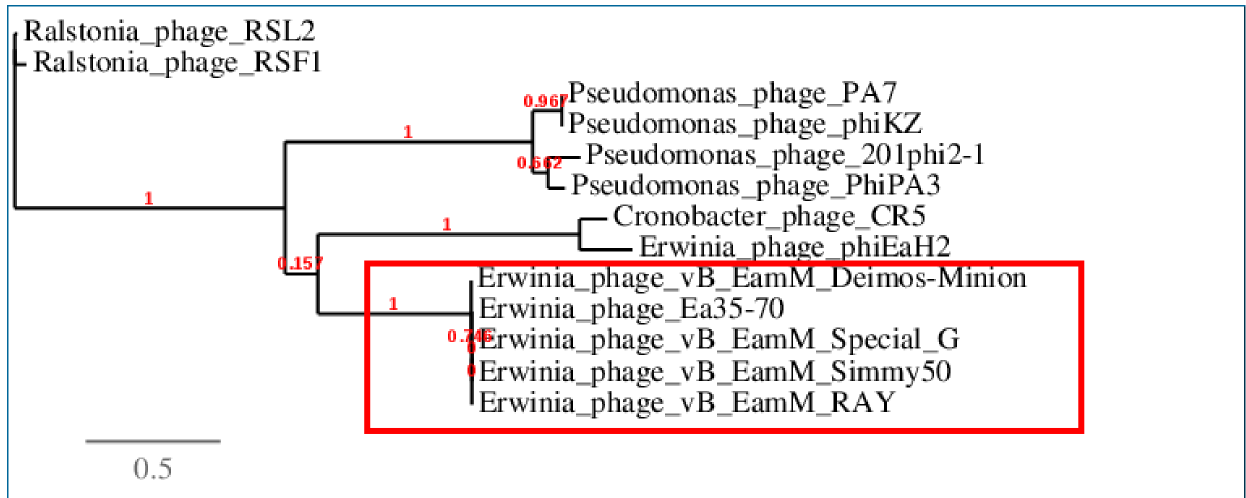

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. Terminase, large subunit

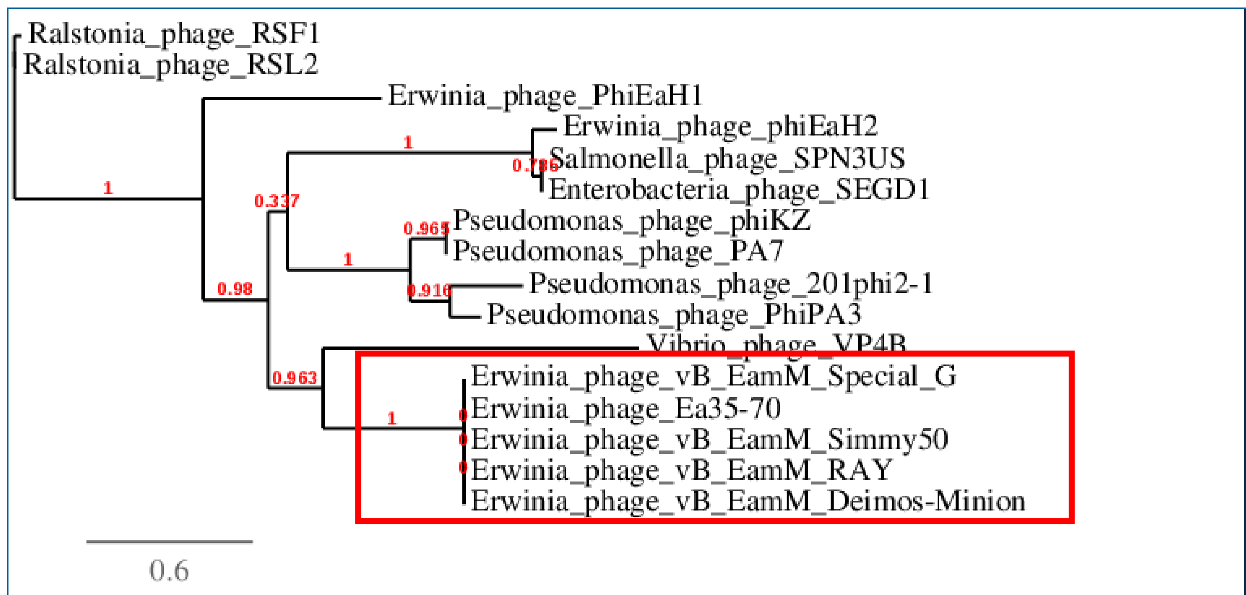

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 4. progressiveMauve alignment [2] of the genomes of Erwinia phages: from top to bottom: Ea35-70, Deimos-Minion, RAY, Simmy50, Special_G. Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity (Aaron Darling, personal communication).